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Antigenic relationship among antihemorrhagic factors from snake and opossum plasmas.

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Brazilian J Med Biol Res (1989) 22: 717-719 *SHORT COMMUNICATION*

ANTIGENIC RELATIONSHIP AMONG ANTIHEMORRHAGIC FACTORS FROM SNAKE AND OPOSSUM PLASMAS

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Immunological relationships among antihemorrhagic factors (AHF) present in the plasma of different snakes and a mammal (opossum) were studied. Antibodies were prepared against purified *Bothrops jararaca* and *Didelphis marsupialis aurita* (opossum) AHF. The antigen-antibody reaction was determined by direct ELISA and by the competition of homologous antigen-antibody reaction with a heterologous competitor antigen. Plasmas from several snakes and from opossum were used as antigens. Anti-AHF *(B. jararaca)* reacted only with snake plasmas. Antibody against opossum AHF was detectable only when the homologous antigen was used. Differences in the reactivity of snake plasmas with antibodies against *B. jararaca* were demonstrable only when a competition assay was used: *B. alternatus* showed a reaction pattern similar to that of *B. jararaca* while *Crotalus durissus terrificus* and the nonvenomous snake, *Wanglerophis merremii*, presented reduced reactivity.

Key words: antigenic relationships of antihemorrhagic factor, snake venoms, opossum antihemorrhagic factor.

Most crotalidae and viperidae venoms cause hemorrhage and it is generally agreed that this activity contributes to their lethality. Some animals, however, are highly resistant to snake venom, a fact that seems to be due to the presence of a protein in their blood which neutralizes the hemorrhagic activity of the venom. Antihemorrhagic factor (AHF) has been described in the blood of snakes (1-3) and some mammals (4-6). It is a relatively low molecular weight protein (50-90 kDa) which migrates in the α_1-albumin region during electrophoresis. The aim of the present study was to determine the antigenic relationships between these factors. We studied the interaction of plasmas from resistant species with antibodies prepared against AHF isolated from either *Bothrops jararaca* or *Didelphis marsupialis aurita* (opossum) plasmas.

AHF was purified from the plasma of *B. jararaca* by three chromatographic processes: DEAE-Sephacel, Phenyl-Sepharose and Biogel P-200 (M.M. Tanizaki, unpublished results). The purified protein, molecular weight 54 kDa, was used to immunize Swiss mice. Groups of mice were injected with 4 weekly doses of 20 μg AHF/mouse in 1 mg/ml $Al(OH)_3$ as adjuvant. Blood was collected 10 days after the last booster and the serum was separated and stored at -20°C.

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Antibodies against the glycoprotein fraction from opossum serum which contained antihemorrhagic activity (7) was kindly donated by Dr. Haity Moussatché, Instituto Oswaldo Cruz, Rio de Janeiro.

Antibody titers were measured by ELISA using the different plasmas as antigens (2 µg/well) and anti-mouse or anti-rabbit IgG peroxidase plus OPD/H_2O_2 as conjugated enzymes and substrate, respectively. The antigens used were plasma from *B. jararaca, B. alternatus, Crotalus durissus terrificus* and *Wanglerophis merremii* as well as the serum from *D. m. aurita* (opossum) all of which strongly inhibited hemorrhage caused by *B. jararaca* venom.

As shown in Table 1, the titer of antibodies against AHF from *B. jararaca* plasma was 12,800 using homologous antigen. Antibodies were not detected when the wells were coated with opossum plasma. Similarly, anti-opossum AHF antibodies were detected only when homologous plasma was used as antigen. Thus, no antigenic relationship was observed between opossum and *B. jararaca* AHF by ELISA. However, titers obtained in the reaction of plasmas from *B. jararaca, B. alternatus, C. d. terrificus* or *W. merremii* against antibodies anti-*B. jararaca* AHF suggest that these components are immunologically related among these snakes.

Table 1 - *Antibody titers of anti-opossum and anti-*B. jararaca *antihemorrhagic factors using homologous and heterologous plasmas as antigens.*

ND, Not detectable using a 1:5 plasma dilution.

Source of plasma	Anti-opossum AHF antibodies	Anti-*B. jararaca* AHF antibodies
B. jararaca	ND	12,800
D. m. aurita	204,800	ND
B. alternatus	ND	25,600
C. d. terrificus	ND	6,400
W. merremii	ND	6,400
Human serum	ND	ND

Table 2 - *Inhibition of the reaction between anti-*B. jararaca *AHF antibodies and* B. jararaca *AHF by homologous and heterologous plasmas used as competitor antigens.*

Data are reported as plasma volume needed to obtain 50% inhibition and the maximum inhibition obtained with undiluted plasma.

Source of plasma	50% Inhibition	Maximum inhibition
B. jararaca	30 nl	100%
D. m. aurita	> 40 µl	35%
B. alternatus	37 nl	100%
C. d. terrificus	1.3 µl	82%
W. merremii	1.4 µl	70%
Human serum	> 40 µl	37%

The ELISA competition assay was performed in order to determine the extent of AHF homology among these snakes. In this assay, each well of the microtitration plates was

coated with 3 nl of *B. jararaca* pla
antigens and a fixed antibody dil
reacted with the fixed antigen was
Table 2, 50% inhibition of the ho
plasma was added as competitor.
was very close to that of homolog
(37 nl). Greater amounts of *C. d*
50% of the homologous reaction (

AHF from *B. jararaca*
with the functionally equivalent
species, AHFs from *B. jararaca* a
indicated by the similarity in the i
contrast, AHFs from *C. d. terrif*
partial antigenic cross-reactivity
undiluted plasmas were used as co

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& Mandelbaum FR (1981). *Acta Cientifi*

*rs of anti-opossum and anti-*B. jararaca *sing homologous and heterologous plasmas*

a 1:5 plasma dilution.

opossum AHF ntibodies	Anti-*B. jararaca* AHF antibodies
ND	12,800
204,800	ND
ND	25,600
ND	6,400
ND	6,400
ND	ND

*ie reaction between anti-*B. jararaca *AHF a AHF by homologous and heterologous r antigens.*

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% Inhibition	Maximum inhibition
30 nl	100%
> 40 µl	35%
37 nl	100%
1.3 µl	82%
1.4 µl	70%
> 40 µl	37%

d. terrificus or *W. merremii* against ponents are immunologically related

. in order to determine the extent of well of the microtitration plates was

coated with 3 nl of *B. jararaca* plasma. After blocking, increasing dilutions of the competitor antigens and a fixed antibody dilution (1:500) were added. The amount f antibodies that reacted with the fixed antigen was determined with anti-mouse IgG-peroxidase. As shown in Table 2, 50% inhibition of the homologous reaction was achieved when 30 nl of *B. jararaca* plasma was added as c mpetitor. The amount of plasma required to provide 50% inhibition was very close to that of homologous plasma when *B. alternatus* plasma was used as inhibitor (37 nl). Greater amounts of *C. d. terrificus* and *W. merremii* plasma were needed to inhibit 50% of the homologous reaction (1.3 µl and 1.4 µl, respectively).

AHF from *B. jararaca* is cross-reactive among snakes but does not cross-react with the functionally equivalent protein present in opossum serum. Within the Bothrops species, AHFs from *B. jararaca* and *B. alternatus* appear to be closely related antigenically as indicated by the similarity in the inhibition of the homologous antigen-antibody reaction. In contrast, AHFs from *C. d. terrificus* or the nonvenomous snake *W. merremii* present only partial antigenic cross-reactivity since 100% inhibition was not achieved even when undiluted plasmas were used as competitors.

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